Monitoring of Sigatoka disease (*Mycosphaerella musicola* LEACH ex MULDER) in the Windward Islands for tolerance to fungicides.

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CERCOSPORIOSE DANS LES WINDWARD ISLANDS : CONTROLE DES TOLERANCES AUX FONGICIDES.

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RESUME - La cercosporiose causée par *Mycosphaerella musicola* LEACH est à ce jour la principale des maladies foliaires dans les Windward Islands et plusieurs autres pays producteurs de bananes. Une méthode simple de surveillance de la tolérance aux fongicides utilisant les conidies de *Cercospora musae* est décrite.
Cette méthode évite une confusion possible que peut entraîner l'observation des ascospores d'autres espèces de *Mycosphaerella*. Aucun résultat n'a révélé de tolérance du pathogène à 5 ppm de bénomyl dans les Windward Islands mais *Mycosphaerella musae* (voisin très commun sur feuille de bananier) est souvent tolérant.

INTRODUCTION

In the Windward Islands, which consist of Dominica, St. Lucia, St. Vincent and Grenada, the major type of banana leafspot is Sigatoka disease caused by *Mycosphaerella musicola* LEACH ex MULDER. The same is presently true for the other islands of the Caribbean, from Cuba to Trinidad, and also in several banana growing territories of South America, Africa and Australia. Black Sigatoka or black leaf streak caused by *M. fijiensis* var. *difformis* MULDER and STOVER and *M. fijiensis* MORELET respectively are now the predominant banana leafspots in Central America and the Pacific Islands and although they already pose a serious threat in varying degrees to the forementioned territories, it is difficult to predict a time of arrival or rate of spread.

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One of the principal phenotypic differences between *M. musicola* and *M. fijiensis* is that the former pathogen produces abundant conidia on easily recognizable sporodochia and these play an important role in the epidemiology of the disease (STOVER, 1970, 1980). *M. fijiensis* produces far fewer conidia on groups of one to eight conidiophores emerging through the stomatal pores (LEACH, 1964), and this pathogen relies to a greater extent on ascospore production for its dissemination.

For the past three years conidia of *M. musicola* have been used in a simple laboratory test to monitor for any tolerance to benzimidazole fungicides in the Windward Islands. Conidia are particularly useful for such a test because they are easy to obtain in the laboratory and there is little chance of confusing their cylindrical shape with any other common spore type found from the banana phyllosphere. Ascospores of *M. musicola* are often used in fungicide tolerance tests but can easily be confused with those of *M. musae*, *M. minima* and *Leptosphaeria* spp., particularly when viewed at a magnification which is

useful for rapid observation on culture plates (ca. x 50). They are only clearly distinguished at ca. x 600 when size differences between *M. musae* and *M. musicola* can be measured, and this is particularly important as *M. musae* is very commonly found on ascospore discharge plates in the Windward Islands. Conidia of *M. musicola* (stat. conid. *Cercospora musae* ZIMM.) are easily recognizable on agar plates at x 50 magnification which makes them convenient to use for tolerance tests.

METHODOLOGY

Samples of diseased leaf lamina containing predominantly stage four spots described by BRUN (1963) were brought fresh to the laboratory. At this stage spots were dark brown to black in colour with a slightly sunken centre. The leaf samples were cut into pieces small enough to fit adaxil surface uppermost into 9 cm Petri dishes containing a filter paper disc of the same diam. Before closing the dishes, water was sprayed over the leaf pieces so that the run off thoroughly wetted the filter paper.

The leaf pieces were incubated for 24 h at room temperature and examined under a dissecting microscope to find spots on which abundant production of conidia had occured. These single spots were carefully cut out, placed adaxil surface downwards onto freshly prepared 2 % water agar plates into which 5 ppm benomyl had been added. The spots were then gently smeared over the surface of the agar so that the conidia were dislodged and spread along the smear. Equal numbers of control plates without benomyl were treated identically. Up to five replicate spots were applied per plate.

The plates were incubated at room temperature for up to 48 h and examined at x50 magnification. At this time some contamination of the smears by bacteria, yeast, or other fungal colonies had occured but they were sufficiently small to allow easy observation of the *C. musae* conidia where they were well separated along the smear.

At the points of placement and removal of the leaf tissue on the agar surface there were generally many spores and contaminants closely crowded making observations more difficult. Incorporation of 0.05 g/l chloramphenicol into the water agar was not found to greatly improve the technique.

Susceptibility of the conidia to the 5 ppm benomyl was observed as a definite inhibition of germination when compared with control plates. Knob-like swellings from one or several cells of the conidia were often present or a hook shaped germ tube which rarely exceeded 10 µ in length.

DISCUSSION

Benzimidazole fungicides (benomyl, thiabendazole and methyl thiophanate) carried in spray oil have been continously used for Sigatoka control in the Windward Islands since 1975. During this time the average number of fungicide applications per year has been eight with a maximum of ten being given to any area. For the last three years spotted leaf samples from areas of high infestation in the islands have been collected and subjected to the conidia germination test described above. All results so far have indicated that *M. musicola* is not tolerant to 5 ppm benomyl in the Windward Islands. Ascospores obtained from leaves on the same plants have given the same results when used in parallel tests.

The conidia germination test is easy to carry out since there is no confusion between spores of different species and conidia are more reliably produced in the laboratory than ascospores. Ascospores of *M. musae* are very commonly found together with *M. musicola* when heavily spotted leaf tissue is allowed to discharge spores onto water agar plates. In addition to the similar appearence of these spores a further possibility for misleading results can occur since ascospores of *M. musae* are often found to be tolerant to 5 ppm benomyl in the Windward Islands. This tolerance is also seen when isolations on mycophil agar containing 10 ppm benomyl are made from diseased leaf tissue.

In this laboratory 5 ppm benomyl is used to indicate tolerance. This concentration is somewhat arbitrary and concentrations as low as 0.1 ppm have been used elsewhere.

There is at present a lack of information on the persistence of fungicidal concentrations of systemic chemicals on and within the leaf tissue following relatively large concentrations applied to the leaf surface. The application rate of 126 g benomyl in 15 l oil per ha used in the Windward Islands gives a concentration of 8400 ppm benomyl deposited on the leaf surface. It would be useful if pesticide manufacturers could provide information with each product of the concentration at which tolerance can be considered to render its use no longer advisable.

Although *M. musicola* has not yet exhibited tolerance to benzimidazole formulations in the Windward Islands, the phenomenum occured in 1977 in Surinam (STOVER, 1979 a) and more recently in the French Antilles (BUREAU et al., 1982). Similarly in 1976 the *M. fijiensis* pathogens first showed tolerance up to 200 ppm benomyl after 2-3 years use in Honduras (STOVER, 1979 a, 1979 b), and then in 1978 in Belize, Guatemala and the Philippines (STOVER, 1979 a), and recently in Western Samoa (FIRMAN, 1982). For this reason fungicides with alternative mode of action to the benzimidazoles have been tested in the Windward Islands (CRONSHAW, 1982). Results showed that imazalil, bitertanol, nuarimol and tridemorph

all gave control of *M. musicola* at least as good as benomyl.

Taking economic factors into consideration an alternation programme of benzimidazole and tridemorph carried in oil is now recommended (CRONSHAW, 1982 b) to reduce the number of benzimidazole applications to 4-5 per year. It is hoped that at this frequency periodic monitoring will not reveal tolerance and that in future it will be possible to introduce other fungicides into the rotation programme.

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